

AMENDMENTS TO THE CLAIMS

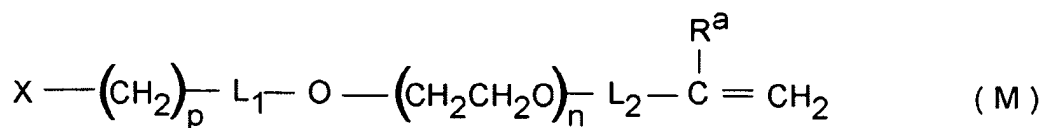
1. (Currently amended) A method to detect an analyte in an aqueous solution with use of an agglutination reaction of polymer-based fine particles dispersed in said solution, which comprises contacting the analyte with the polymer-based fine particle, wherein:

(a) said fine particle has, as a core, a polymer chain segment with a chargeable group-carrying recurring unit, and has, as plural brushes on said core or as a shell, ~~nonionic-a~~ hydrophilic polymer chain or segment of ~~said-the~~ hydrophilic polymer chain, wherein a member of a biologically specific bond which forms a counterpart to the analyte is bound to at least a part of free terminals of said hydrophilic polymer chain,

(b) the agglutination reaction is conducted under a condition under which the fine particles can be bonded to the analyte or absorb the analyte to form agglutinated matter, and, subsequently, the agglutinated matter is treated under a condition of a raised ionic intensity, under which, although the biologically specific bond between the fine particles is not cleaved, a bond made by electrostatic interaction can be cleaved, and

(c) the existence of agglutinated matter which remains after the treatment of step (b) is detected by a method capable of distinguishing a state in which the biologically specific bond is not cleaved, and a state in which the bond by electrostatic interaction is cleaved, the results being used as an index of the presence of analyte,

wherein the hydrophilic polymer chain in the polymer-based fine particles is formed from a poly(ethylene glycol) macro monomer having formula (M):



wherein X denotes a hydrogen atom, -COOM group (M denotes a hydrogen atom or an organic group), -CHR¹R² (R¹ and R² either independently denote a C₁₋₆ alkyloxy group, phenyloxy group or a phenyl-C₁₋₃ alkyloxy group, or, taken together, denote -OCHR'-CH₂O- wherein R' denotes a hydrogen atom or a C₁₋₆ alkyl group) or -CH=O, R^a denotes a hydrogen atom or a C₁₋₆ alkyl group,

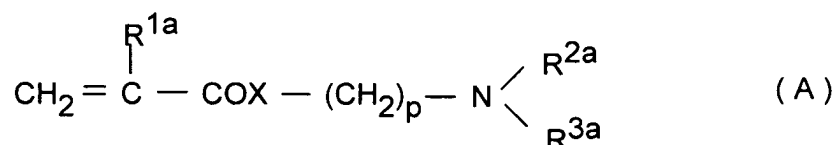
L₁ denotes a methylene group or a carbonyl group.

L₂ denotes a carbonyl group, a C₁₋₃ alkylene group or a C₁₋₃ alkylphenylene group,

n denotes an integer of 2 to 10,000, and

p denotes an integer of 1 to 5; and

wherein the polymer chain with a recurring unit carrying a chargeable group in the polymer-based fine particles is formed from a monomer having a formula (A):

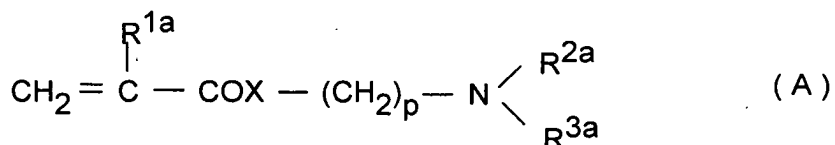


wherein R^{1a} denotes a hydrogen atom or a C₁₋₆ alkyl group, R^{2a} and R^{3a} either, independently, denote a C₁₋₆ alkyl group or, taken together, may form, with the nitrogen atom to which they are bound, a five- or six-membered heterocycle which may contain further one or two nitrogen atoms, an oxygen atom or a sulfur atom. X denotes -O- or -NH-, and p denotes an integer of 2 to 6; and

wherein said two monomers are copolymerized with a crosslinking agent and/or an ethylenically polymerizable group-containing diluting monomer to give a random copolymer, said crosslinking agent and diluting monomer being allowed, where necessary, to be mixed with each other before crosslinked.

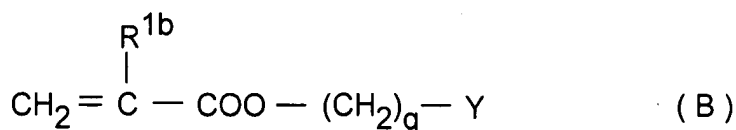
2. (Previously presented) The method of claim 1, wherein the chargeable group in the polymer-based fine particles is selected from the group consisting of tertiary amino group, secondary amino group, carboxyl group, sulfo group and phosphono group.

3. (Currently amended) The method of claim 1, wherein the ~~nonionic~~ nonionic-hydrophilic polymer chain in the polymer-based fine particles is originated in a polymer which is selected from the group consisting of polyethylene glycol, poly(vinyl alcohol), poly(vinyl pyrrolidone) and poly(N,N-dimethylacrylamide), and wherein the polymer chain carrying a chargeable group in the polymer-based fine particles is either composed of a monomer having a formula (A):

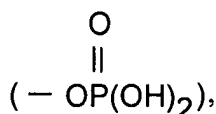


wherein R^{1a} denotes a hydrogen atom or a C_{1-6} alkyl group, R^{2a} and R^{3a} either, independently, denote a C_{1-6} alkyl group or, taken together, may form, with the nitrogen atom to which they are bound, a five- or six-membered heterocycle which may contain further one or two nitrogen atoms, an oxygen atom or a sulfur atom, X denotes $-O-$ or $-NH-$, and p denotes an integer of 2 to 6;

or is composed of a monomer having a formula (B):



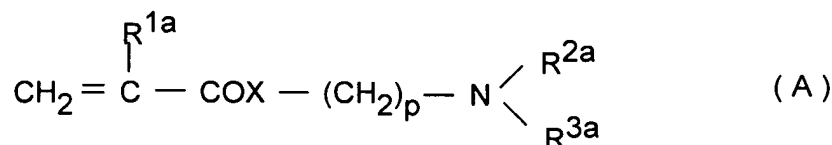
wherein R^{1b} denotes a hydrogen atom or a C_{1-6} alkyl group, Y denotes carboxyl group ($-\text{COOH}$), sulfo group ($-\text{SO}_3\text{H}$), oxysulfo group ($-\text{OSO}_3\text{H}$) or oxyphosphono group



and q denotes an integer of 0 to 4, provided that, when q is 0, Y denotes a hydrogen atom; or is composed of a polymer selected from the group consisting of poly(lysine), poly(3- ω -N,N-di C_{1-6} alkylamino- C_{2-4} alkyl aspartate), poly(4- ω -N,N-di C_{1-6} alkylamino- C_{2-4} alkyl glutamate), poly(aspartic acid) and poly(glutamic acid).

4. (Previously presented) The method of claim 1, wherein the hydrophilic polymer chain in the polymer-based fine particles is originated in polyethylene glycol, and

wherein the polymer chain with a recurring unit carrying a chargeable group is composed of a monomer having a formula (A):



wherein R^{1a} denotes a hydrogen atom or a C_{1-6} alkyl group, R^{2a} and R^{3a} either, independently, denote a C_{1-6} alkyl group or, taken together, may form, with the nitrogen atom to which they are bound, a five- or six-membered heterocycle which may contain further one or two

nitrogen atoms, an oxygen atom or a sulfur atom, X denotes $-O-$ or $-NH-$, and p denotes an integer of 2 to 6.

5. (Previously presented) The method of claim 4, wherein the hydrophilic polymer chain segment and the polymer chain segment with a recurring unit carrying a chargeable group in polymer-based fine particles constitute a block copolymer.

6. (Cancelled)

7. (Currently amended) ~~The method of claim 1,~~ A method to detect an analyte in an aqueous solution with use of an agglutination reaction of polymer-based fine particles dispersed in said solution, which comprises contacting the analyte with the polymer-based fine particle, wherein:

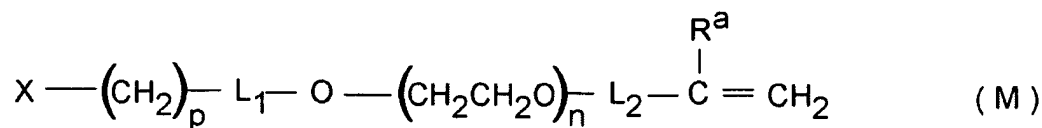
(a) said fine particle has, as a core, a polymer chain segment with a chargeable group-carrying recurring unit, and has, as plural brushes on said core or as a shell, a hydrophilic polymer chain or segment of the hydrophilic polymer chain, wherein a member of a biologically specific bond which forms a counterpart to the analyte is bound to at least a part of free terminals of said hydrophilic polymer chain,

wherein the polymer-based fine particles have, encapsulated in their core domain, an ultrafine particle of inorganic material which is selected from the group consisting of semiconductor, free electron metal, magnetic material and silica,

(b) the agglutination reaction is conducted under a condition under which the fine particles can be bonded to the analyte or absorb the analyte to form agglutinated matter, and, subsequently, the agglutinated matter is treated under a condition of a raised ionic intensity, under which, although the biologically specific bond between the fine particles is not cleaved, a bond made by electrostatic interaction can be cleaved, and

(c) the existence of agglutinated matter which remains after the treatment of step (b) is detected by a method capable of distinguishing a state in which the biologically specific bond is not cleaved, and a state in which the bond by electrostatic interaction is cleaved, the results being used as an index of the presence of analyte,

wherein the hydrophilic polymer chain in the polymer-based fine particles is formed from a poly(ethylene glycol) macro monomer having formula (M):



wherein X denotes a hydrogen atom, -COOM group (M denotes a hydrogen atom or an organic group), -CHR¹R² (R¹ and R² either independently denote a C₁₋₆ alkyloxy group, phenyloxy group or a phenyl-C₁₋₃ alkyloxy group, or, taken together, denote -OCHR'-CH₂O- wherein R' denotes a hydrogen atom or a C₁₋₆ alkyl group) or -CH=O,

R^a denotes a hydrogen atom or a C₁₋₆ alkyl group,

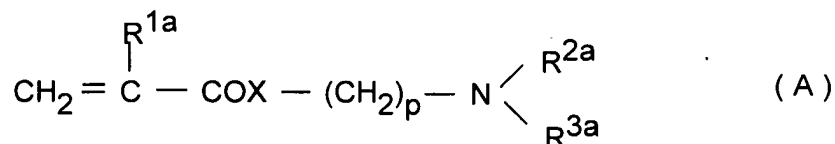
L₁ denotes a methylene group or a carbonyl group,

L₂ denotes a carbonyl group, a C₁₋₃ alkylene group or a C₁₋₃ alkylphenylene group,

n denotes an integer of 2 to 10,000, and

p denotes an integer of 1 to 5; and

wherein the polymer chain with a recurring unit carrying a chargeable group in the polymer-based fine particles is formed from a monomer having a formula (A):



wherein R^{1a} denotes a hydrogen atom or a C₁₋₆ alkyl group, R^{2a} and R^{3a} either, independently, denote a C₁₋₆ alkyl group or, taken together, may form, with the nitrogen atom to which they are bound, a five- or six-membered heterocycle which may contain further one or two nitrogen atoms, an oxygen atom or a sulfur atom, X denotes -O- or -NH-, and p denotes an integer of 2 to 6; and

wherein said two monomers are copolymerized with a crosslinking agent and/or an ethylenically polymerizable group-containing diluting monomer to give a random copolymer, said crosslinking agent and diluting monomer being allowed, where necessary, to be mixed with each other before crosslinked.

8. **(Previously presented)** The method of claim 4, wherein the polymer-based fine particles have, encapsulated in their core domain, an ultrafine particle of semiconductor.
9. **(Previously presented)** The method of claim 1, wherein one of companion pieces of the biologically specific bond is one of antibody and its antigen or hapten; one of receptor protein and lectin, hormone and neurotransmitter which are to bond the receptor protein; one of streptavidin and biotin derivative; and one of enzyme and its substrate.
10. **(Previously presented)** The method of claim 1, wherein the condition of a raised ionic intensity is putting the agglutinated matter under a high concentration of salt.
11. **(Previously presented)** The method of claim 1, wherein the condition of a raised ionic intensity is adjusting the concentration of salt to 0.1 to 2 M.
12. **(New)** The method of claim 7, wherein one of companion pieces of the biologically specific bond is one of antibody and its antigen or hapten; one of receptor protein and lectin, hormone and neurotransmitter which are to bond the receptor protein; one of streptavidin and biotin derivative; and one of enzyme and its substrate.
13. **(New)** The method of claim 7, wherein the condition of a raised ionic intensity is putting the agglutinated matter under a high concentration of salt.
14. **(New)** The method of claim 7, wherein the condition of a raised ionic intensity is adjusting the concentration of salt to 0.1 to 2 M.